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Expression and location of IL-17A, E, F and their receptors in colorectal adenocarcinoma: comparison with benign intestinal disease

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ABSTRACT

The research aimed to investigate secretion, expression and location of IL-17 relative ligands, IL-17 relative receptors, infiltrating inflammatory cells and parenchymal

structural cells in colorectal cancer (CRC) compared with ulcerative colitis (UC) and benign hyperplastic polyp. 29 human intestinal tissues with CRC, 17 with UC and 7 with polyp were stained using immunohistochemistry to evaluate immunoreactivity for IL-17 family relative ligands including IL-17A, E, F and their respective relative receptors such as IL-17RA, IL-17RB and IL-17RC. At the same time the infiltration of inflammatory cells including lymphocytes, phagocytes, mast cells and neutrophils and parenchymal structural cell changes involving vascular endothelial cells and CD90⁺ fibroblast cells were also evaluated using the same methods. The immunoreactivity or positive inflammatory cells of all the sections were analyzed using professional image analysis software to determine statistical significance. The immunoreactivity for IL-17A, IL-17RA, IL-17E, IL-17RB and IL-17F showed significant decrease in CRC tissue when compared to UC ($p = 0.00001$, respectively). The reduction of above IL-17 relative ligands and receptors was accompanied by an obvious decrease in the number of infiltrating neutrophils and mast cells in CRC ($p = 0.00001$ and $p = 0.007$, respectively) but accompanied by a marked increase of CD31⁺ blood vessels ($p = 0.001$). The immunoreactivity of IL-17A, IL-17RA, IL-17E, IL-17RB and IL-17F and the numbers of infiltrating neutrophils and mast cells showed significant decrease in CRC tissues when compared to those in polyp ($p < 0.05$). In contrast, the immunoreactivity of IL-17RC and the numbers of CD3⁺ lymphocytes were elevated in CRC when compared with those in polyp ($p = 0.0001$, $p = 0.007$, respectively). In CRC tissues, positive correlations between IL-17A, IL-17RA with CD68⁺ macrophages were observed respectively ($r = 0.621$, $p = 0.0001$;

$r = 0.75$, $p = 0.0001$). IL-17 cytokine family including ligands and their corresponding receptors were secreted and expressed by infiltrating inflammatory cells. Not only infiltrating lymphocytes but also increased blood endothelial cells were relative significantly to genesis and progression of CRC.

Keywords: IL-17 cytokine family, colorectal adenocarcinoma, ulcerative colitis, inflammatory cell, structural cell.

1. Introduction

In worldwide, colorectal cancer (CRC) is among the top three causes of cancer related death in men and women. According to the statics, there are about 50,000 patients died from CRC each year. Sessile serrated adenomas/polyps and ulcerative colitis (UC) are recognized as risk factor for CRC especially of larger, multiple or familial polyps and chronic UC [1-3]. The pathogenesis of UC, polyp and CRC is not very clear, but many factors especially cytokines and inflammatory mediators are related to the occurrence and development of those intestinal diseases. Previous research showed that cytokine family such as IL-17 ligands and their receptors signals participated in occurrence and development of those diseases [4, 5].

The IL-17 family plays important roles in solid tumor research involving prostate cancer, bladder cancer, lung cancer and glioma etc and becomes one of the hot spots in recent years. IL-17 family is consists of six ligands including interleukin 17

(IL-17A) interleukin 17B (IL-17B), interleukin 17C (IL-17C), interleukin 17 D (IL-17D), interleukin 17E, (IL-17E) and interleukin 17F (IL-17F) and their respective relative receptors IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE, which share sequence homology like their ligands. The binding of ligands and their receptors affect the gene expression of downstream through complex pathways, which can produce different biological effects. Among all of the IL-17 family ligands, IL-17A and IL-17E are the most concerned cytokines because they share opposite biological functions in the field of solid tumor [6, 7]. In addition, IL-17F has also become research hotspot because it has high molecular homology with IL-17A and possess similar functions to IL-17A especially in tumor field [6]. The collected data confirmed that IL-17A mainly connected with its receptor IL-17RA and IL-17E mainly binds to its receptor IL-17RB to produce different biological functions in malignant tumor fields. IL-17F can also bind to IL-17RA or IL-17RC to regulate the expression of its downstream genes which promotes tumor formation or progression like IL-17A-IL-17RA signal [8]. Because of high degree homology, IL-17A and IL-17F share similar functions and are main pro-inflammatory cytokines in inflammatory intestinal diseases and malignant tumors [9]. While IL-17E (also called IL-25) which has relatively low homologous sequences with IL-17A and IL-17F integrates with its own receptor exerting significant biological effect in asthma and atopic diseases [10]. In addition, IL-17E has been proved to inhibit human melanoma xenograft growth *in vivo*[11].

Previous studies have reported IL-17A, IL-17F and other cytokines are produced and secreted mainly by monocytes (including Th17 lymphocytes, macrophage and eosinophils, etc.) and secondly by structural cells (such as smooth muscle and endothelial cells). Those cytokines contribute to the pathogenesis of UC and CRC [4,12], however, we do not know the expression location and expression amount of IL-17 family cytokines, the infiltrating numbers of inflammatory cells and the changes of structural cells in CRC, benign polyp and UC. We also don't know whether there is a correlation between IL-17 family cytokines with occurrence and progression of intestinal diseases. So we systematically compared immunoreactivity of IL-17 family ligands and their relative receptors, infiltrating inflammatory cells and structural cells using immunohistochemistry in those tissues from polyp, UC and CRC. In order to explore the relationship between those diseases and above indicators, we analyzed correlations between IL-17 family cytokines themselves, the infiltrating inflammatory cells and the changes of structural cells with IL-17 family cytokines in CRC.

2. Materials and methods

2.1. Patients and specimens

The written informed consents were obtained from the First Hospital of Jilin University (NO. JL20130218), the Affiliated Hospital of Beihua University (NO. BH20130014) and the People's Hospital of Jilin City (JLS2013006), Jilin Province,

People's Republic of China, from January 2011 to December 2013. We obtained human colon specimens from methods to processes in accordance with the Medical Ethical Principles Involving Human Subjects, which were formulated in the World Medical Association Declaration of Helsinki (revised in 2008). The blocks included 29 patients with colorectal adenocarcinomas, 17 patients with UC and 7 patients with colorectal hyperplastic polyps. All of the patients with adenocarcinomas, 26 tumors were carried out surgical resection and 3 tumors were endoscopic biopsy and the other intestinal tissues were endoscopic biopsy. Serial tissue sections with 4 μ m were cut for each patient sample. The first serial section was stained for H& E, and the diagnostic criteria were described as previously [13-15]. The remaining sections were stained with specific antibodies using Immunohistochemical staining. The summary of patients and clinical data were showed in Table 1.

2.2. Immunohistochemistry

All the tissue slides were stained with a similar protocol as previously described [16]. Antigen retrieval was realized under high temperature and high pressure with sodium citrate solution (PH=6). Endogenous peroxidase activity was inhibited using hydrogen peroxide in methanol (0.3%) at room temperature for half an hour. Slides were washed with PBS and then blocked with 2.5% horse serum blocking buffer (Vector Laboratories, Cat# S-2012) for 20 minutes, and then with dilution buffer with goat serum (5%) for 30 minutes. All washes and blocking steps were at room

temperature. We observed the secretion, expression and location for IL-17 family relative cytokines, inflammatory cells (such as neutrophils, lymphocytes, macrophages and mast cells) and structural cells (for example CD90⁺ fibroblasts and vascular endothelial cells) as previously described [16]. We used the specific antibodies, which were against CD3 (lymphocyte), CD68 (macrophage), elastase (neutrophil), tryptase (mast cell), CD31 (mainly vascular endothelial cell) and CD90 (mainly fibroblast) (Table 2) to identify infiltrating inflammatory cells and parenchymal structural cell types. Specific information about primary antibody and their optimal dilutions are summarized in Table2. We used DAB kit (diaminobenzidine, ZhongShan Golden Bridge Biological Company, Beijing, China) to detect positive signals.

All the slides with immunohistochemical staining including IL-17 family relative ligands and receptors, CD90⁺ fibroblasts, infiltrating inflammatory cells and CD31⁺ blood vessel were scanned at 10× magnification using an Olympus microscope. These images were exported as TIFF files and uploaded into Image Pro Plus 6.0 software (Media Cybernetics, Maryland, USA). Separate investigators were proficiency in the software applications and absolutely agreed with manual hand counts. The positive signals were quantified as the percentage staining of the total stainable area of the sections for IL-17 family cytokines and CD90⁺ cells by the haematoxylin counterstaining, while infiltrating inflammatory cells and CD31⁺ blood vessels were quantified as the numbers of cells per unit area of the entire sections as before [16].

2.3. Statistical analysis

Data were analyzed using a commercially available statistical package (Minitab for Windows, Minitab Release 9.2; Minitab, Inc, State College, PA) provided by Dr. Sun Ying. Differences between groups were analyzed using Kruskal-Wallis test followed by the Mann-Whitney U test. Data are presented in the figures as the mean \pm SEM. A p value of less than 0.05 was considered statistically significant. The correlation between IL-17 family relative cytokines with inflammatory cells and structural cells was performed using a non-parametric Spearman correlation.

3. Results

3.1. Secretion and expression of IL-17A and its receptor IL-17RA in intestinal tissues

The IHC results indicated that IL-17A immunoreactivity was significantly higher in UC and polyp samples compared with CRC (Fig 1A-B, $p = 0.00001$ and $p = 0.001$, respectively). In addition, its expression amount was significantly higher in polyp samples compared with UC samples ($p = 0.012$). Mononuclear cells in interstitium, glandular epithelium, vascular endothelial cells and some malignant cells could secrete IL-17A.

The IHC results indicated that IL-17RA expression amount was also significantly higher in UC and polyp samples compared with CRC (Fig 1C-D, $p = 0.0001$ and $p = 0.001$, respectively), while there was no significant statistical difference between UC and polyp samples ($p = 0.567$). The expression amount of IL-17RA immunoreactivity was similar to IL-17A (Fig 1C-D), which was also predominantly expressed in mononuclear cells in interstitium, glandular epithelium, vascular endothelial cells as well as some malignant cells.

3.2. Secretion and expression of IL-17E and its receptor IL-17RB in intestinal tissues

Our results indicated that IL-17E located mainly in non-tumor tissues and cells. In UC and polyps samples IL-17E immunoreactivity was significantly higher compared with CRC (Fig 2A-B, $p = 0.00001$ and $p = 0.0005$, respectively). While there was no obvious differences for IL-17E immunoreactivity between UC and polyp samples (Fig 2A-B, $p = 0.998$). IL-17E immunoreactivity mainly located in mononuclear cells in interstitium, glandular epithelium and some vascular endothelial cells.

IL-17RB, the receptor of IL-17E, was secreted by mononuclear cells, epithelial cells and vascular endothelial cells (Fig 2C). Its immunoreactivity was higher in UC samples compared with polyp ($p = 0.022$) and CRC ($p = 0.00001$) (Fig 2C-D).

Compared with CRC samples, IL-17RB expression amount in polyp samples was also significantly elevated ($p = 0.0001$).

3.3. Secretion and expression of IL-17F and its receptor IL-17RC in intestinal tissues

Our IHC results showed that positive IL-17F was significantly higher in UC and polyp samples compared with CRC (Fig 3A-B, $p = 0.00001$ and $p = 0.0007$, respectively), but there was no significant difference for IL-17F immunoreactivity between UC and polyp samples (Fig3A-B, $p = 0.423$). Similar to IL-17A and IL-17E, IL-17F was secreted principally by mononuclear cells, epithelium, vascular endothelial cells as well as some malignant cells (Fig 3A).

Previous research data indicated that IL-17F not only combines with IL-17RA but also with IL-17RC to produce biological effect, so we detected IL-17RC expression amount in all the intestinal samples. Our research results indicated that IL-17RC was expressed on mononuclear cells, epithelium, vascular endothelial cells and some malignant cells in CRC (Fig 3C). Its immunoreactivity was significantly reduced in polyp compared with UC ($p = 0.003$) and CRC ($p = 0.0001$), while no significant difference was observed between UC and CRC samples ($p = 0.195$).

3.4. Infiltration of T lymphocytes and macrophages in intestinal tissues

CD3⁺ cells, as the marker for lymphocytes, infiltrated mainly in interstitium of UC, polyp and CRC. Compared with polyp, the number of cells stained with this cellular marker was higher in both UC ($p = 0.019$) and CRC ($p = 0.007$) (Fig 4A-B), while there was no significant difference in number of positive cells in UC and CRC ($p = 0.792$).

CD68⁺ cells, as the marker for macrophages, infiltrated mainly in interstitium. Through analysis, there were not obvious differences in number of CD68⁺ positive cells among the three groups (Fig 4C-D, UC vs CRC: $p = 0.776$; polyp vs CRC: $p = 0.110$, and UC vs polyp: $p = 0.168$, respectively).

3.5. Infiltration of neutrophils and mast cells in intestinal tissues

Because under ordinary circumstances, a large number of *E. coli* bacteria grow in the gut, we also detected other related inflammatory cells infiltration. Elastase⁺ neutrophils and tryptase⁺ mast cells were detected in the sections from UC, polyp and CRC through IHC staining. The results showed both neutrophils and mast cells infiltrated mainly in interstitium. In UC tissues the median number of neutrophils was higher compared with polyp and CRC (Fig5A-B, $p = 0.01$ and $p = 0.00001$, respectively), while we did not observe significant differences in number of neutrophils between polyp and CRC ($p = 0.615$).

The median numbers of mast cells per unit area of the sections in UC and polyp were significantly higher than that in CRC (Fig 5 C-D, $p = 0.007$ and $p = 0.001$, respectively), while no significant differences in number of mast cells were seen between polyp and UC ($p = 0.427$) (Fig5C-D).

3.6. Changes in structural cells in intestinal tissues

As we know IL-17 family cytokines were secreted and expressed not only by monocytes (lymphocytes, phagocytes, neutrophils and mast cells) but also by other parenchymal cells (e.g vascular endothelial cells and glandular epithelium etc), so we detected parenchymal cell changings including vascular endothelial cells and CD90⁺ fibroblast cells. The number of CD31⁺ vascular endothelial cells increased in polyp and CRC compared with UC (Fig 6A-B, $p = 0.0001$, $p = 0.001$), we did not observe significant differences in number of blood vessels between polyp and CRC samples ($p = 0.12$).

CD90⁺ cells, as the marker of fibroblast, were slightly elevated in CRC compared with UC and polyp, but no significant differences of fibroblasts were seen among the three groups (Fig 6C-D).

3.7. Correlation of IL-17 family relative ligands and receptors, inflammatory cellular infiltration and parenchymal cells changes in CRC

The Pearson correlation method was used to determine the association between immunoreactivity for the IL-17 family ligands and their receptors, inflammatory cells and parenchymal cells in CRC. The obvious positive correlation was observed between IL-17A with its own receptor IL-17RA (Fig 7A, $r = 0.553$, $p = 0.02$); but unfortunately, the correlations between IL-17E with its receptor IL-17RB, IL-17F with its receptor IL-17RC were not identified. Furthermore, the associations between the numbers of CD68⁺ macrophages in CRC tissues were consistent with IL-17A (Fig 7B $r = 0.621$, $p = 0.001$) and IL-17RA (Fig 7C, $r = 0.75$, $p = 0.001$) respectively, which indicated that IL-17A and IL-17RA were mainly secreted and expressed by macrophages.

4. Discussion

Inflammation especially during chronic infection predisposes to cancer initiation and development. In CRC, the imbalance of T helper 1/T helper 2 immune response induces cell proliferation and leads to phenotype change of immune cells in the tumor microenvironment, then a large number of abnormal cytokines will be synthesized and released [17]. During all the cytokines, IL-17 family has become a hot spot of tumor research. In our previous research, we have known that IL-17 family relative cytokines participate in the pathogenesis and the progression of benign prostatic hyperplasia and prostate cancer [16]. The collected data proved that IL-17 family may

UC, polyp and CRC [18-19], but there has no systematically compared study of the IL-17 family relative cytokines (IL-17A, IL-17E, IL-17F, IL-17RA, IL-17RB and IL-17RC) in these common intestinal disorders till now. In this present study, we analyzed the expression and location of IL-17 family relative ligands and their respective receptors from patients with UC, polyp and CRC in these intestinal tissues. Because IL-17 family relative cytokines are secreted and expressed by both mononuclear cells and parenchyma cells, we also simultaneously detected relative inflammatory cells quantity and location, parenchymal structural changes and angiogenesis in order to carry out research of cellular and animal level experiments in the near future.

Because a large number of bacteria live in the intestinal environment, pervasive inflammatory cells (lymphocyte, phagocyte, neutrophil and mast cell, etc.) infiltration is a typical feature in intestinal tract. From our histological tissues we confirmed that more monocytes infiltrated in UC and CRC slides. Such severe infiltration of many inflammatory cells could trigger effective and specific inflammatory response, which conversely synthesizes and eliminates more abnormal cytokines and humoral factors. These abnormal cytokines and humoral factors have double-faced effects, which make further efforts to induce more severe inflammation response, tissue damage even tumorigenesis, tumor growth and metastasis[20]. Therefore, through effective methods to reduce inflammatory cell infiltration and subsequent inflammatory response in intestinal tissues will play an important role in prevention and treatment of cancer.

Our data revealed that the secretion of IL-17A/E/F ligands and the expression of their corresponding receptors IL-17RA/RB/RC increased obviously in UC compared with CRC (Fig 1-3) with distinct infiltration of neutrophils and mast cells between UC and CRC simultaneously (Fig 5), which means that the infiltration of neutrophils and mast cells have some relationship with IL-17 family relative cytokines. From our previous research tissues in prostatic and present data in intestinal tissues we knew that elevated IL-17A/E/F and their receptors contributed to more inflammatory cells infiltration, then these inflammatory cells secreted more cytokines and humoral factors (including IL-17 family ligands and receptors) to cause further inflammation response in intestinal tract. This is a typical positive feedback process. These elevated abnormal cytokines may promote the aberrant cellular proliferation associated with polyp and CRC generation. But the exact mechanism should be investigated further.

Through comparative analysis we knew that secretions of IL-17A/E/F and their expressions of receptors were markedly reduced in CRC compared with UC and polyp besides IL-17CR (Fig 1-3). Perhaps mononuclear cells such as lymphocytes, phagocytes, mast cells, neutrophils and eosinophiles and other structural cells such as smooth muscles and glandular epithelial cells synthesized and secreted IL-17 relative family cytokines. In our histological sections, more inflammatory cells infiltrate in UC tissue and a large amount of glandular epithelial cells proliferate in polyp tissue, while In CRC tissue the mononuclear cells (especially neutrophils and mast cells) were lower (Fig 5) which explained the intrinsic link between them partly. Additionally, IL-17RC expression was elevated with damaged and/or abnormal

structural cells in CRC, which indicated that colorectal cancer cells can expressed IL-17C and participated in the pathogenesis of colorectal cancer with unknown pathways. Both IL-17A and IL-17F can combine with IL-17RC to induce biological responses, while IL-17F is rather important although we are not clear of it and its receptor IL-17RC in the pathogenesis of CRC. The collected data showed IL-17F can provoke angiogenesis because elevated microvessel density in turn contribute to IL-17A or IL-17F production and release [21]. IL-17A and IL-17F are cytokines with strong proinflammatory effects and their increased expression are often found in malignant tumors [22-24]. The positive feedback cycle in intestinal tissues will promote cancerous cell proliferation even metastasis. Interestingly, the secretion of IL-17F decreased but the expression of IL-17RC increased in CRC compared with UC and polyp in our study, suggesting that IL-17F and IL-17RC are possibly secreted and expressed not by single cell population. Until far, there is lack of study investigating whether malignant cells can express more IL-17F receptor in CRC although it has been shown that the IL-17F genetic polymorphisms correlate with the risk of CRC in Chinese people [25]. The functions of IL-17F-IL-17RC signal in intestinal tumor microenvironment are complex and their relationship with CRC has not been elucidated yet. But targeting IL-17F or IL-17RC using different methods might provide a new treatment for CRC. In order to figure out the roles of IL-17 relative family cytokines on intestinal relative diseases, further experiments are needed to perform *in vivo* and *in vitro*.

Previous studies have demonstrated that IL-17A and IL-17F positive cells are

frequently present in CRC. Both IL-17A and IL-17F have the properties to promote occurrence and progression of malignancy when combined with their receptors, while IL-17E has different properties and acts on a distinct receptor IL-17RB, as well as IL-17RA to exert anti melanoma effects [26]. Al-Samadi A et al showed that IL-17B was increased in CRC with a strong presence both in the epithelial and stromal compartments, IL-17C showed different expression depending on the grade of differentiation and IL-17E remained unchanged compared to healthy colons. In contrast, IL-17F was decreased in CRC [27]. In the present study, IL-17E increased in both UC and polyp but reduced in CRC. Our research results indicated that IL-17E also participated in the pathogenesis of UC and polyps. IL-17E might involve in the inflammation response through acting on its receptor IL-17BR or IL-17RA expressed on mononuclear cells such as lymphocytes, phagocytes and mast cells or structural cells such as fibroblast cells, smooth muscles and blood vessels then resulting in different degrees of inflammation and changings of structural cells [3,10,13,26]. On the other hand, we observed that IL-17E and receptor (IL-17RB) decreased in CRC samples (Fig 2). The expression of IL-17RB in CRC also decreases, which indicates that the infiltration of mononuclear cells is less since these cells can also secret and release IL-17RB [13]. On the other hand the results suggest that IL-17E-IL-17RB signal transduction plays anti tumor role in CRC. If there are any, the details and underlying mechanisms of IL-17E signal in the pathogenesis of CRC should be explored and targeting this signal through different methods will create satisfactory biological effects.

Angiogenesis plays a significant role in the development and spread of colorectal cancer and has been recognized as a prognostic marker in a variety of human neoplasms [28]. More vascular endothelial cells were observed in CRC and polyp compared with UC from these intestinal tissues (Fig 6). As we know that adequate blood supply is one of the essential conditions for tumor survival. More angiogenesis with adequate blood is a very important link from tumor growth, progression to metastasis in CRC, because cancer cells need more blood supplying compared with normal or benign tumor to sustain its abnormal cell proliferation, conversely these cancerous cells and elevated angiogenesis might promote other cell aberrant proliferation and induce changes in tumor microenvironment. This possibly explains at least in part the reason why the number of CD31⁺ blood vessels increased and numbers of monocytes such as neutrophils and mast cells decreased in CRC.

Inflammatory cell infiltration is one of the typical characteristics of the intestinal tract especially in UC, while IL-17 family cytokines closely link with inflammation. Although IL-17A is a weak NF- κ B activator, when synergize with other cytokines like TNF- α it can promote and prolong pro-inflammatory responses [22]. We accordingly detected inflammatory cell types through immunohistochemistry staining with different specific antibodies in our study. Tumor-infiltrating lymphocytes are the main actors in suppressing and controlling the host immune response to tumor cells. However, the detailed regulatory mechanisms remain to be elucidated.

Inflammatory infiltration is typical characteristics in intestinal relative diseases. Our research showed that the numbers of CD3⁺ T lymphocytes and CD68⁺ macrophage

increased in UC and CRC tissues (Fig 4), which suggests that these cells might involve in occurrence and development process of UC and CRC. Again, macrophage is an essential component of tumor microenvironment and plays an important role in tumor progression especially tumor-associated macrophages. Macrophage has promoting or inhibiting tumor dual function in different tumor types [29]. So the function of macrophage is very complex and should be elucidated in the following research. The increased infiltrating macrophages in UC and CRC were significantly higher compared with polyp, which indicated that these macrophages might play an important role through secreting different numbers of cytokines or humoral factors in enteritis genesis and in CRC progression.

In present study, we observed that IL-17A has positive correlation with its receptor IL-17RA in CRC (Fig 7), simultaneously, both markers were also positively associated with CD68⁺ macrophages (Fig 7), which suggested that IL-17A and IL-17RA were mainly synthesized and secreted by CD68⁺ macrophages. Significances of such pathways of IL-17A and its receptor IL-17RA on macrophages in CRC should be further discussed in the following research.

Although we have done a series of comparative research in IL-17 family cytokines, inflammatory cells infiltration and parenchymal cells changings in intestinal tissues, there exists some limitations in present study. Firstly, we have not collected the complete normal samples, and the samples from patients were not enough, which make the conclusion questionable. Secondly, despite immunohistochemistry staining was applied to detect specific antigen expression and

location in UC, polyp and CRC pathological tissues, we do not know for certain if this method is accurate because it is not so specific. Finally, the specimens were obtained through endoscopic biopsy or resection in our study, which has some limitations. All of these may bias to verify the results.

In summary, our data suggest that IL-17 family cytokines may participate in the pathogenesis of CRC and other intestinal disorders such as UC and polyp in distinct pathways. Inflammatory cells infiltration and structural changings all promote occurrence and progression in CRC as well as UC and polyp. Targeting or promoting these cytokines or inflammatory cells may provide alternative therapeutic benefits for clinic treatment.

In summary, our research data suggest that infiltrating inflammatory cells especially phagocytes might be associated with IL-17A and IL-17RA, while infiltration of lymphocytes and increased blood endothelial cells might be associated with CRC occurrence and development.

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Figure Legends

Figure. 1. Secretion and expression of IL-17A and IL-17RA in UC, polyp and CRC tissues. **A:** The staining with the marker for IL-17A in intestinal sections from subjects with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29) (original magnification x 10 and 20, Arrows representing positive staining). **B:** Quantitative analysis of IL-17A positive area of intestinal sections (% of whole sections). **C:** The staining with the marker for IL-17RA in intestinal sections from UC, polyp and CRC (original magnification x 10 and 20, Arrows representing positive staining). **D:** Quantitative analysis of IL-17RA positive area of intestinal sections (% of whole sections). Data are expressed as the mean \pm SEM.

Figure. 2. Secretion and expression of IL-17E and IL-17RB in UC, polyp and

CRC tissues. A: The staining with the marker for IL-17E in intestinal sections from subjects with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29) (original magnification x 10 and 20, Arrows representing positive staining). **B:** Quantitative analysis of IL-17E positive area of intestinal sections (% of whole sections). **C:** The staining with the marker for IL-17RB in intestinal sections from UC, polyp and CRC (original magnification x 10 and 20, Arrows representing positive staining). **D:** Quantitative analysis of IL-17RB positive area of intestinal sections (% of whole sections). Data are expressed as the mean \pm SEM.

Figure. 3. Secretion and expression of IL-17F and IL-17RC in UC, polyp and CRC tissues. A: The staining with the marker for IL-17F in intestinal sections from subjects with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29) (original magnification x 10 and 20, Arrows representing positive staining). **B:** Quantitative analysis of IL-17F positive area of intestinal sections (% of whole sections). **C:** The staining with the marker for IL-17RB in intestinal sections from UC, polyp and CRC (original magnification x 10 and 20, Arrows representing positive staining). **D:** Quantitative analysis of IL-17RC positive area of intestinal sections (% of whole sections). Data are expressed as the mean \pm SEM.

Figure. 4. Infiltration of CD3⁺ T cells and CD68⁺ macrophages in UC, polyp and CRC tissues. A and C: Representative immunohistochemistry images of CD3⁺ lymphocytes and CD68⁺ macrophages in intestinal sections from subjects with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29)

(original magnification x 10 and 20, Arrows representing positive cells). **B** and **D**: Quantitative analysis of numbers of CD3⁺ T cells and CD68⁺ macrophages per unit area of intestinal sections. Data are expressed as the mean \pm SEM.

Figure. 5. Infiltration of elastase⁺ neutrophils and tryptase⁺ mast cells in UC, polyp and CRC tissues. **A** and **C**: Representative immunohistochemistry images of elastase⁺ neutrophils and tryptase⁺ mast cells in intestinal sections from subjects with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29) (original magnification x 10 and 20, Arrows representing positive cells). **B** and **D**: Quantitative analysis of numbers of elastase⁺ neutrophils and tryptase⁺ mast cells per unit area of intestinal sections. Data are expressed as the mean \pm SEM.

Figure. 6. Expression for CD90⁺ fibroblasts and CD31⁺ blood vessels in UC, polyp and CRC tissues. **A** and **C**: Representative immunohistochemistry images of CD31⁺ blood vessels and CD90⁺ fibroblasts with ulcerative colitis (UC, n = 17), polyp (n = 7) and colorectal cancer (CRC, n = 29) (original magnification x 10 and 20, Arrows representing positive cells). **B**: Representative immunohistochemistry images and quantitative analysis of numbers of CD31⁺ blood vessels in intestinal sections. **D**: Quantitative analysis of CD90⁺ fibroblast positive area of intestinal sections (% of whole sections). Data are expressed as the mean \pm SEM.

Figure. 7. Correlations between IL-17A, IL-17RA and CD68⁺ macrophages in

CRC. A: There existed obvious positive correlation between IL-17A and IL-17RA. B: Positive correlation was found between IL-17A expression and infiltration of CD68⁺ macrophages. C: Positive correlation was found between IL-17RA expression and infiltration of CD68⁺ macrophages.

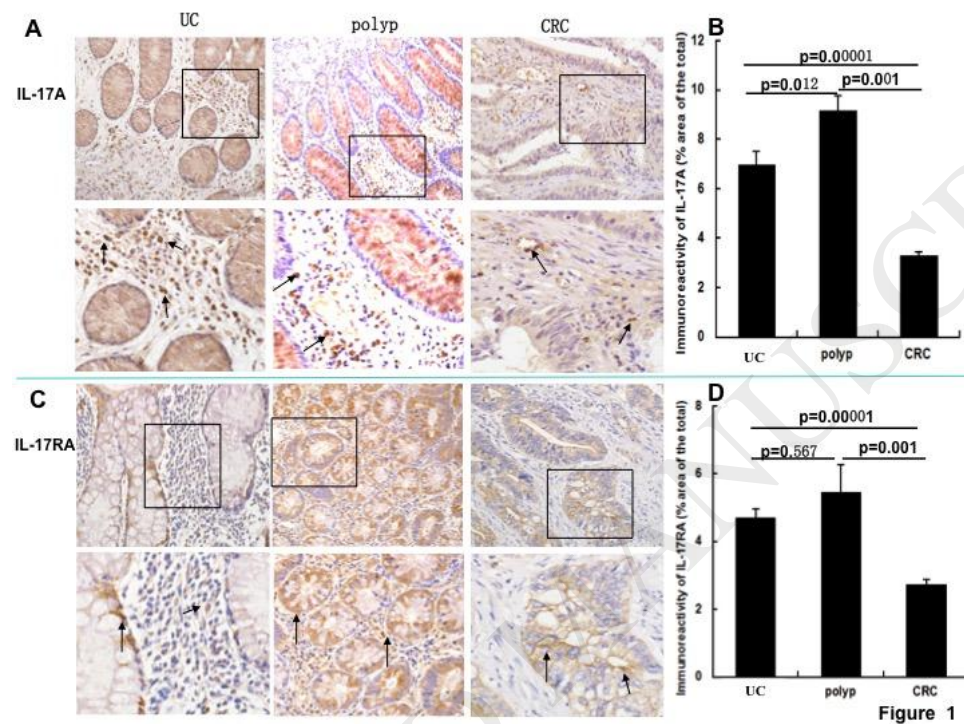


Figure 1

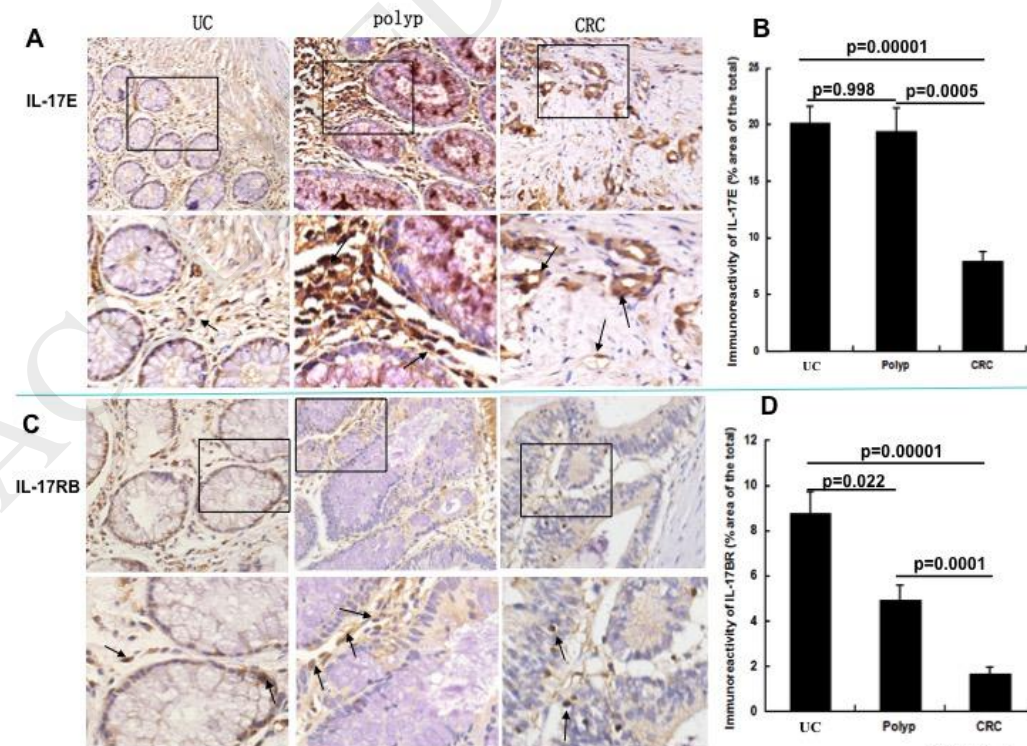


Figure 2

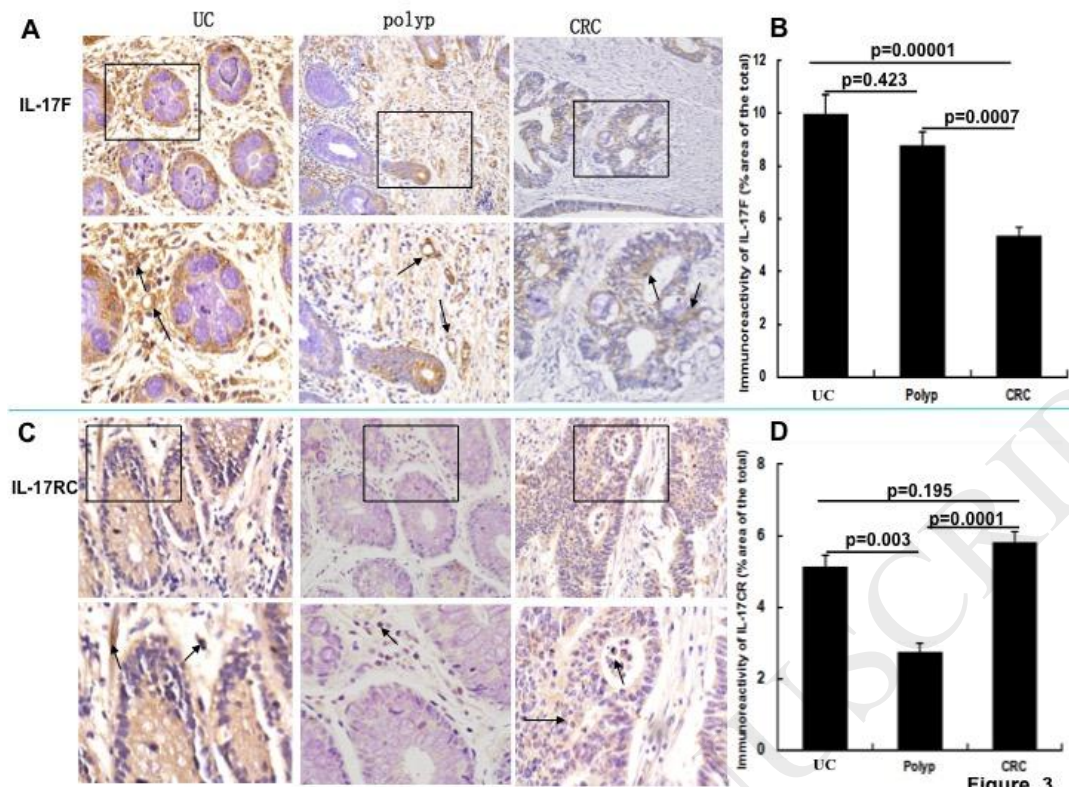


Figure 3

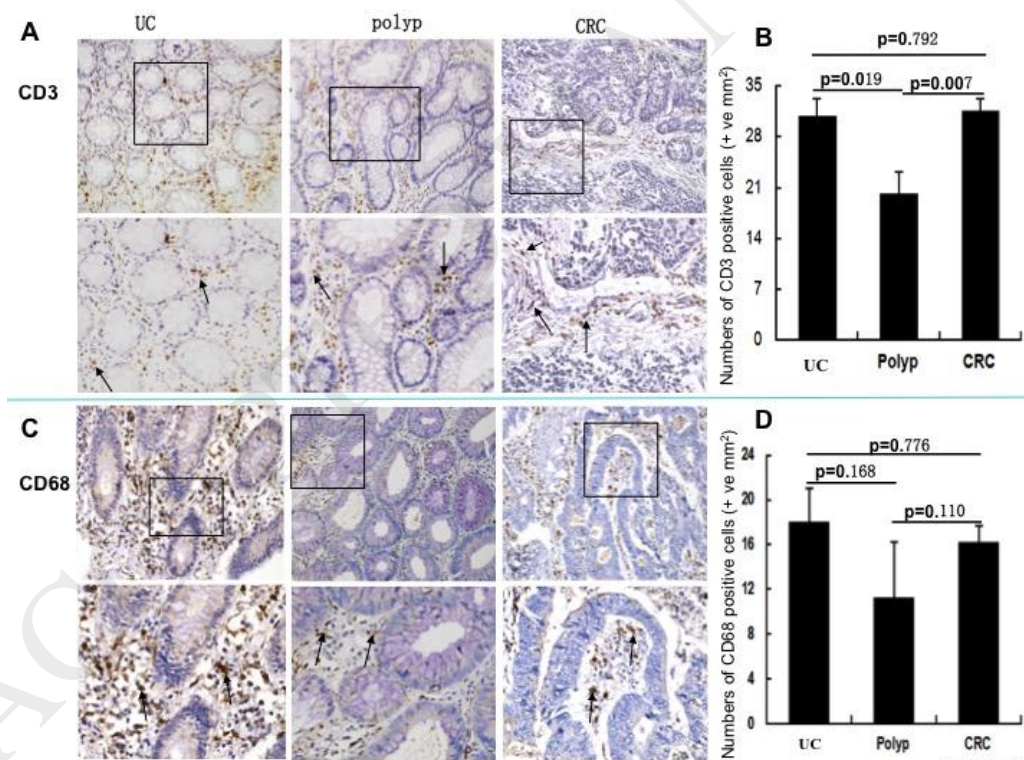
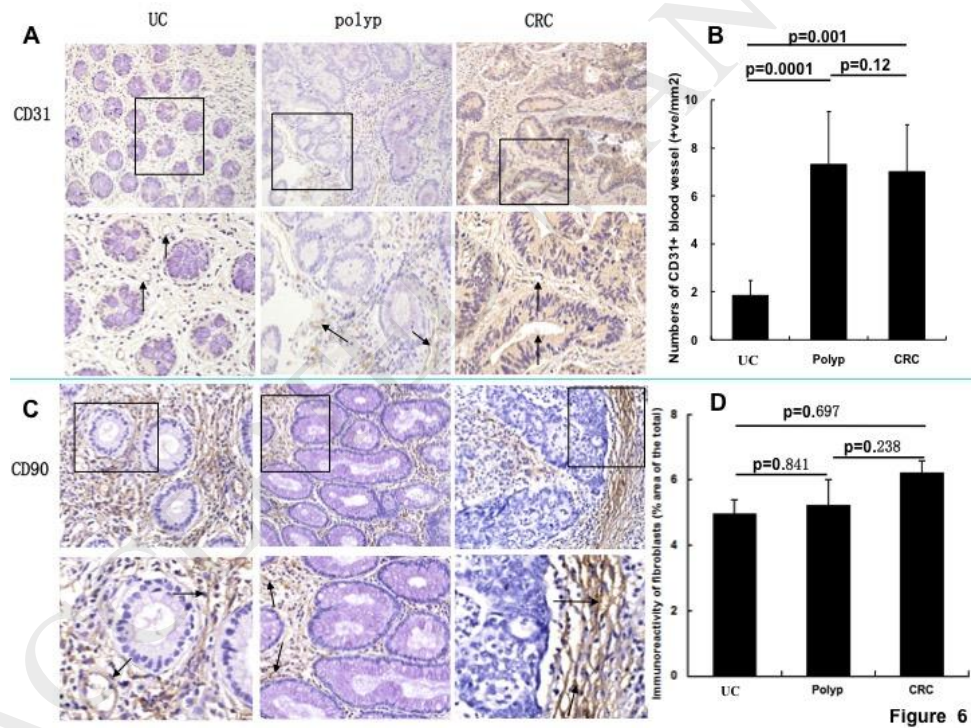
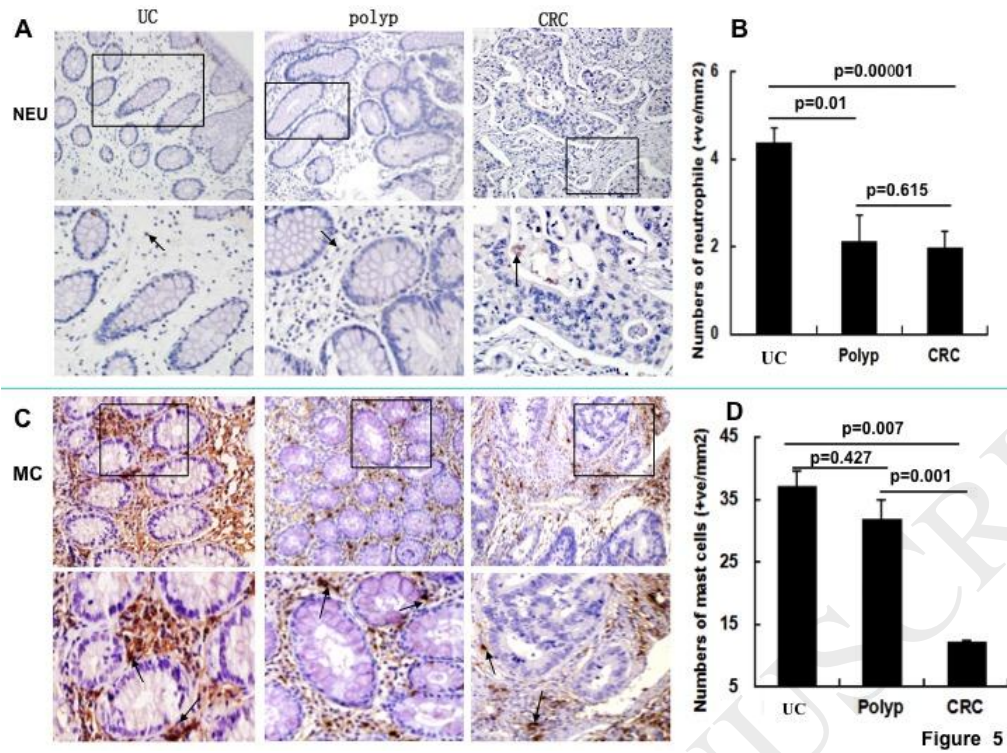


Figure 4



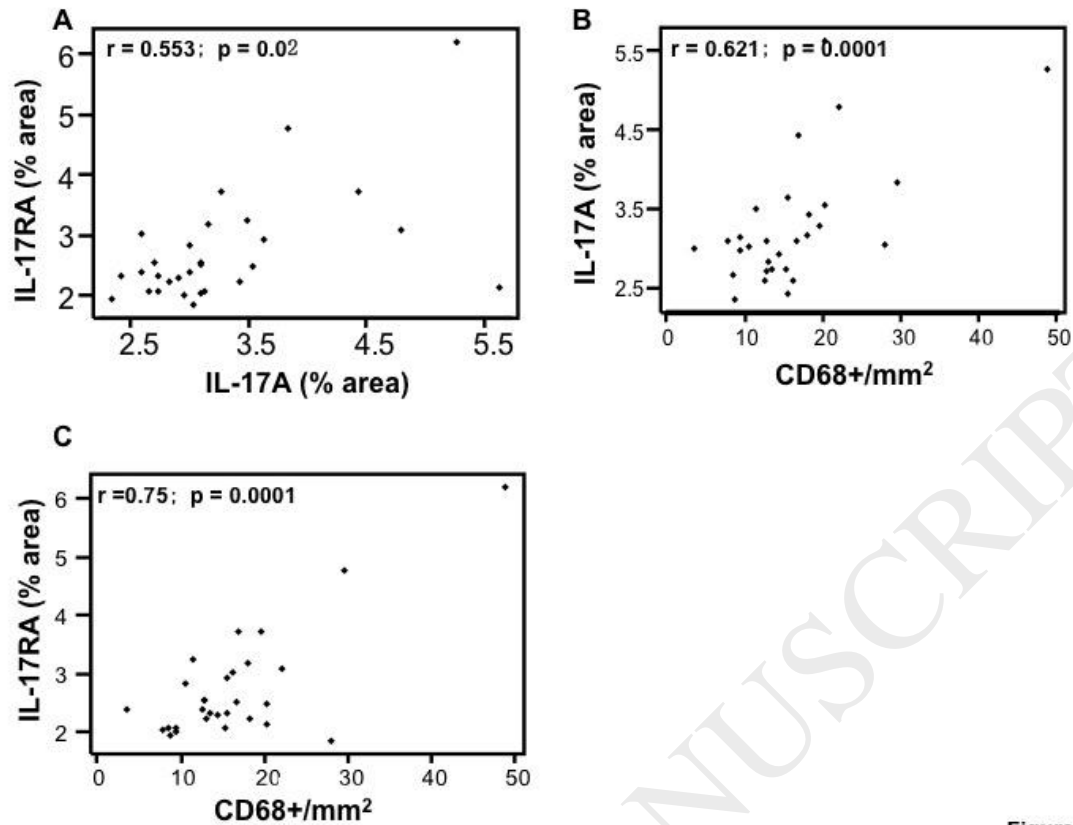


Figure 7

Table 1

Clinical characteristics of the subjects

Status	Age (median)	Sources	Pathological characteristics	Differentiation grade
Ulcerative colitis (UC) n=17	52.8 (32-75) Male 13 Female 4	Endoscopic biopsies		
Polyp n=7	54.3(22-84) Male 6 Female 1	Endoscopic biopsies or resection specimens	Hyperplastic polyp	
Colorectal cancer (CRC) n=29	64.5 (43-83) Male 24 Female 5	Endoscopic biopsies or resection specimens	Adenocarcinoma	High (n=8) Middle (n=17) Low (n=4)

Table 2

Relative antibodies used in this research

Antibody	Item number	Isotype	Dilution	Sources
Anti-IL-17A	NBP1-42746	Rabbit-IgG	1:300	Novus iologicals (USA)
Anti-IL-17E	NB100-56541	Mouse-IgG1	1:4000	Novus Biologicals (USA)
Anti-IL-17F	NBP2-21684	Mouse-IgG1	1:1000	Novus Biologicals (USA)
Anti-IL-17RA	ab133416	Goat-IgG	1:200	Abcam (Hong Kong) Ltd (China)
Anti-IL-17RB	NBP1-39952	Mouse-IgG1	1:200	Novus Biologicals(USA)
Anti-IL-17RC	NBP1-83112	Rabbit-IgG	1:50	Novus Biologicals(USA)
PECAM-1 (Anti-CD31)	SC-53411	Mouse-IgG1	1:20	Santa Cruz Inc (USA)
Anti-CD90/Thy1	NBP1-42068	Rabbit-IgG	1:100	Novus Biologicals(USA)
Anti-CD3	ab699	Mouse-IgG2a	1:20	Abcam (Hong Kong) Ltd (China)
Anti-CD68	sc-70761	Mouse-IgG	1:800	Santa Cruz Inc (USA)
Anti-Neutrophil Elastase	Ab21595	Rabbit-IgG	1:800	Abcam (Hong Kong) Ltd (China)
Anti-Mast Cell Tryptase	Ab64192	Rabbit-IgG	1:2000	Abcam Ltd (USA)